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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US96/00493 (22) International Filing Date: 11 January 1996 (11.01.96) (30) Priority Data: 08/369,233 6 January 1995 (06.01.95) US (71) Applicant: IGEN, INC. [US/US]; 16020 Industrial Drive, Gaithersburg, MD 20877 (US). (72) Inventor: TALLEY, David; 18258 Paladin Drive, Olney, MD 20832 (US). (74) Agents: RYAN, John, W. et al.; Igen, Inc., 16020 Industrial Drive, Gaithersburg, MD 20877 (US).		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: ELECTROGENERATED CHEMILUMINESCENCE THROUGH ENHANCED PARTICLE LUMINESCENCE (57) Abstract <p>An emission enhancing particulate support is disclosed where the optical properties of the support and the emissive characteristics of a metal luminescent tag are matched to provide enhanced luminescence in electrogenerated chemiluminescent assays.</p>		

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Title of the Invention
ELECTROGENERATED
CHEMILUMINESCENCE THROUGH
ENHANCED PARTICLE LUMINESCENCE

5 Field Of Invention

 The present invention relates to
luminescent metal tagged supports and their use
in assay, sequencing and separation
applications. In particular, the present
10 invention relates to a solid phase support that
provides reproducible surface presentation with
enhanced light emission characteristics as a
result of optical matching with a luminescent
tag and used in immunoassays and DNA probe
15 assays via electrogenerated chemiluminescent
techniques.

Background Of Invention

 Diagnostic techniques such as
immunoassays, DNA probe assays and, fluorescent
20 and separation assays frequently involve a solid
phase support or substrate. The supports, often

in particulate form, provide sites for antibodies, antigens and probes, that may or may not be tagged. The supports maybe be inorganic, such as siliceous based glasses, or they may be
5 polymeric in composition, such as polystyrene.

The solid phase support may contain metallic particles, such as iron oxide (Fe_2O_3), that are coated with a material that provides appropriate sites for adhering the tagged
10 component that aids in determining the analyte of interest. For example, polymeric or siliceous coatings will be applied. Instead of a coating, a particulate polymeric or siliceous material will incorporate Fe_2O_3 to provide the
15 magnetic properties. The magnetic content allows the support to be more easily concentrated and /or separated. Porous bodies of magnetic glass or crystals containing magnetic crystals are also known. The glass
20 materials serve as a site for immobilizing an antibody, antigen, protein or probe etc. The magnetic properties allow the particles to be used in assays or reactors and be easily separated therein. See for example U.S. Patent
25 Nos. 3,042,543 to Schuele, U.S. Patent No. 4,233,169 to Beall et al., 4,631,211 to

Houghten, 4,812,512 to Buendia et al., 5,037,882 to Steel, 5,141,813 to Nelson and 5,128,204 to Charmot.

5 In PCT Application WO 93/10162 to Wong, magnetic porous inorganic supports are prepared for use in chromatography, immunoassays, synthesis, separation and purification techniques. The porous material of Wong has a particle size on the order of 1 to
10 about 200 μ with a specific pore size. A variety of moiety components such as enzymes, antibodies, antigens, peptides, nucleotides, saccharides or cells are bonded to the support surface.

15 It is also known that enzyme, antibody, antigen, peptide, nucleotides, saccharide or cell complements of the analyte of interest can be tagged with conventional tagging agents such as radioactive, colorimetric and/or
20 luminescing agents. Of particular interest are labels which can be made to luminesce through photochemical, chemical, or electro-chemical reaction schemes. In particular, electro-chemiluminescent methods of determining the
25 presence of labelled materials are of interest for the insensitive, nonhazardous and inexpensive properties of the technique.

Present electrochemiluminescent methods use organic compounds and metal chelates. For example, Leland et al. in J. of Electrochemical Society, Vol. 137, No. 10, 5 October 1990 report an ECL reaction between amines, such as, tripropylamine and $\text{Ru}(\text{bpy})_3^{2+}$ (bpy 2,2'-bipyridine). It is proposed that upon electrochemical oxidation of both a luminophore and coreactant amine a strong emission is 10 observed. Use of $\text{Os}(\text{bpy})_3^{2+}$ is also taught by Leland et al. Electrochemiluminescent ruthenium- and osmium-containing labels have been used for detecting and quantifying analytes of interest in liquid media (U.S. Patent Nos. 15 5,310,687; 5,238,808; and 5,221,605). In addition, electrogenerated chemiluminescent (ECL) measurements to detect solution phase DNA intercalated with ruthenium-containing labels have been described (Carter, M.T. et al. (1990) 20 Bioconjugate Chem 2:257-263). However, detection of solution phase analytes is diffusion controlled and has several drawbacks relative to detection of analytes on solid surfaces. Some of the advantages for solid 25 phase techniques as opposed to solution techniques are: (1) greater sensitivity (detection of monolayer quantities); and (2)

easier support separation. These luminescent systems are of increasing importance in diagnostics.

In U.S. Pat. No. 4,372,745,
5 chemiluminescent labels are used in immunochemical applications where the labels are excited into a luminescent state by reaction of the label with H_2O_2 and an oxalate. However, though the system offers versatility, it lacks
10 selectivity or specificity because typical biological fluids containing the analyte of interest also contain a large number of potentially luminescent substances that can cause high background levels of luminescence.

15 Thus, a need exists for a solid phase system and method that (1) avoids the drawbacks of a diffusion controlled system; (2) is selective; (3) provides a light transmission enhancing support; and (4) provides for
20 reproducible contact with an electrode. The present invention overcomes the limitations and drawbacks of the prior art.

Summary Of Invention

The present invention enhances the
25 emission characteristics of solid phase particles that are used in immuno and DNA probe

assays and other applications. The solid phase particles of glass or polystyrene of the present invention have a specified geometry to provide reproducible presentation of the particles to an electrode surface where they are concentrated. In addition, light transmitting characteristics of the particles are matched with the emission characteristics of an appropriate tag to enhance the transmission characteristics of the particle and therefore maximize the light transmission to the photodetector. The solid phase particles contain a covering or layer of a ruthenium- or osmium-containing chemiluminescent labeled ligand component on the surface thereof that will complex with an analyte of interest. The degree of coating is such that the particle surface is not completely saturated with the ligand-tag component. The light generated during electrogenerated chemiluminescent reaction is detected to determine the analyte of interest.

Thus, an object of the present invention is improve light emission characteristics of solid phase-particulate supports for use in immuno and DNA probe assays.

An aspect of the present invention is to provide a complexible solid phase support

formed from an optically suitable substrate with an exterior layer of a ruthenium or osmium containing label. The ruthenium or osmium label is reacted with a coreactant in the presence of
5 an electrode to generate a detectable emission. The optically suitable substrate provides reproducible presentation of the substrate to an electrode surface to facilitate emission.

A further aspect of the present
10 invention is to provide a solid phase support that enhances luminescence in ECL environments via surface geometry and particle transparency.

An object of the present invention is to detect an analyte of interest by providing a
15 solid phase particle support containing a layer of bound luminescent metal label. The label complexes an analyte of interest and is attracted to or collected on an electrode and thereafter reacted with a coreactant via
20 electron transfer from the electrode to emit a detectable emission.

These and other objects will become more apparent from the following detailed description and drawings.

Brief Description Of The Drawings

- Fig. 1 shows a tagged particle of the present invention in contact with an electrode.
- 5 Fig. 2 shows a tagged particle that lacks surface geometry for reproducible presentation.
- Fig. 3 shows another embodiment of the present invention with a tagged particle providing reproducible presentation.
- 10 Fig. 4 shows an analysis system for gravity separation.
- Fig. 5 shows a flow cell for use with magnetically separable particles according to the present.
- 15 Fig. 6 shows the electrode arrangement for ECL analysis.
- 20

Detailed Description Of Invention

The present invention provides enhanced particle luminescence by designing a particle with (1) geometry that provides
5 reproducible presentation of the particle surface to an electrode; and (2) optical properties matched with the luminescent tag. By optical properties is meant that the particle must be transparent to facilitate light emitted
10 light during the electrochemiluminescent reaction and be matched to the emissive characteristics of the tag. It was unexpected that matching the optical properties of the solid phase support with the emissive
15 characteristics of the luminescent tag would provide the results obtained.

The preferred solid phase particulate support of the present invention includes soda lime glass or polystyrene beads, with or without
20 magnetic particles. The particle geometry must present a reproducible surface to an electrode. Fibrous or particulate material that maximizes surface contact is preferred, including but not limited to particles that are spherical,
25 ellipsoid, oval in shape can also be used. Solid phase particles that are irregular in shape or are aggregates do not provide

acceptable presentation and do not enhance the emission and luminescence of the reaction. Also, uniformity in shape provides for improve concentration.

5 With reference to figure 1, a solid phase support particle 3 formed from polystyrene containing Fe_2O_3 , or soda lime glass, can be collected on electrode 1 by a magnet (not shown) or by gravity. Particles containing Fe_3O_4 are
10 also contemplated. The polystyrene beads are Dynabeads™ M-450, available from Dynal A.S., and are uncoated, uniform and magnetic with -OH resistance. They have a mean diameter of $4.5 \mu\text{m}$ (C.V. max 5%); s.g. = 1.5; magnetic
15 susceptibility - 10^{-2} cgs units; and surface area of about $3-5 \text{ m}^2/\text{g}$. Uniform glass microspheres, available from Duke Scientific Corporation are available in sizes of 1.5 to 40μ . The exterior surface of the bead contacts surface 2 of the
20 electrode 1 at contact point 6. The particle 3 contains a plurality of ligand containing luminescent metal tags 4, although only one is shown for clarity. The size and surface geometry of particle 3 permits electrons from
25 electrode 2 to flow through the bead to provide the necessary electron transfer, i.e., activation for the reaction.

In figure 2, an irregular shaped particle 7 has point contacts 8 that contact surface 2 of electrode 1. The point contacts do not necessarily provide the same degree of particle surface exposure as that of a spherical particle or bead. In addition, the irregularly shaped solid phase support particles precludes reproducible presentation of its surface to the electrode 1.

Figure 3 is an alternative embodiment where the bead 9 is elliptical in shape and provides reproducible presentation of the surface, though to a lesser degree than a spherical bead.

The size of the particulate support is also important because of the effective field distance of the electrode. It is believed that the electron transfer from the electrode to the support is most effective at a distance of up to about 100Å from the surface of the electrode. The mean diameter of the particles suitable for the present invention between about 0.25 to about 10μ, preferably about 1 to 4.5μ. Particle size is one of the many factors that contribute to the luminescent enhancing support of the present invention.

The metal luminescent tag of the present invention is preferably a ruthenium or osmium containing compound and may have polydentate ligands or one or more monodentate
5 ligands. A wide variety of these ligands are known to the art. Polydentate ligands of either ruthenium or osmium include aromatic and aliphatic ligands. Suitable aromatic polydentate ligands include aromatic
10 heterocyclic ligands. Preferred aromatic heterocyclic ligands are nitrogen-containing, such as, for example, bipyridyl, bipyrazyl, terpyridyl, and phenanthrolyl. If the metal chelate has greater than one polydentate ligand,
15 the polydentate ligands may be the same or different.

Suitable polydentate ligands may be unsubstituted, or substituted by any of a large number of substituents known to the art.
20 Suitable substituents include for example, alkyl, substituted alkyl, aryl, substituted aryl, aralkyl, substituted aralkyl, carboxylate, carboxaldehyde, carboxamide, cyano, amino, hydroxy, imino, hydroxycarbonyl, aminocarbonyl,
25 amidine, guanidinium, ureide, sulfur-containing groups, phosphorous containing groups, and the carboxylate ester of N-hydroxysuccinimide.

Suitable monodentate ligands include, for example, carbon monoxide, cyanides, isocyanides, halides, and aliphatic, aromatic and heterocyclic phosphines, amines, stibines, and arsines. A more complete list of the ligands, e.g., monodentate and polydentate ligands, and methods of preparing that can be used in the present invention are set forth in U.S. Patent Nos. 5,310,687, 5,238,808 and 5,221,605, the subject matter of which are incorporated herein by reference. A preferred metal luminescent compound is 4-methyl-4-N-succinimidyloxycarbonylpropyl-2,2-bipyridine Ru(II) hexafluorophosphate.

According to the present invention, the metal label is excited by exposing the solid phase supported metal label chelate to electrochemical energy. The potential at which the oxidation of the metal label will occur depends upon the exact structure of the metal label as well as factors such as the co-reactant utilized, the pH of the solution and the nature of the electrode used. Examples of suitable co-reactants which, when incubated with the solid phase supported metal label chelate in the presence of the electrochemical energy, will result in emission, include tripropylamine (TA),

oxalate or other organic acid such as pyruvate, lactate, malonate, tartrate and citrate. This oxidation can also be performed chemically, with some strong oxidants such as PbO_2 or a Ce(IV) salt.

Those of ordinary skill in the art recognize how to determine the optimal potential and emission wave length of an electrochemiluminescent system. The electrochemiluminescent species may be measured by emitted electromagnetic radiation. For example, the rate of energy inputted into the system can provide a measure of the luminescent species. The measurements can be made either as continuous, rate-based measurements, or as cumulative methods which accumulate the signal over a long period of time. An example of rate-based measurements would be by using photomultiplier tubes, photodiodes or phototransistors to produce electric currents proportional in magnitude to the incident light intensity. Examples of cumulative methods are the integration of rate-based data, and the use of photographic film to provide cumulative data directly.

All of these luminescence-based methods entail repeated luminescence by the

ruthenium-containing compound. The repetitive nature of the detectable event distinguishes these labels from radioactive isotopes or bound chemiluminescent molecules such as luminol. The latter labels produce a detectable event only once per molecule (or atom) of label, thereby limiting their detectability.

Figures 4 and 5 exemplify systems that are suitable for electrogenerated chemiluminescence. In figure 4, gravity separation is relied upon. In the figure, an open vessel 21 contains an electrode 24 with a surface 25 upon which a metal tag containing luminescent support 23 will be collected or concentrated will rest. The bottom of the vessel may be sloped (not shown) to facilitate collection of the tagged supports 23 in solution 22 on the electrode surface 25. If supports 23 contain Fe_2O_3 , a magnet 26 may be used.

In figure 5, a solution containing a metal luminescent tagged support 37, or a sandwiched support 39 formed from the reaction of, for example, an antibody-antigen pair, is fed through tube 32 of flow cell 31. The supports 37 and 39 are attracted to electrode 35 and maintained in position by magnet 34. Energy from electrode 35 across the surface 36 to the

main support provides the energy for the reaction. If the device of figure 5 is used for gravity separation, the electrode 35 is located at bottom wall 38 (shown in broken lines).

5 The following examples illustrate various aspects of the invention but are in no way intended to limit the scope of the invention.

Example A

10 45 μ l of a DMSO solution of 4-methyl-4-N-succinimidyloxy-carbonylpropyl-2,2-bipyridine Ru(II) hexafluorophosphate (0.5 mg in 62.5 μ l) was added to a stirred solution of murine IgG in aqueous Physiologic Buffered Saline (182 μ l of 1
15 mg/ml IgG to 318 μ l PBS). The solution was incubated on a rotator at room temperature for approximately 30 minutes. The coupling reaction was stopped by adding 25 μ l of a 2M glycine
20 solution. The metal labelled antibody was separated from unreacted antibody and derivitized ruthenium using a Sephadex G-25 column. The labelled antibody product was analyzed spectrophotometrically.

Example B

To determine a degree of coating that does not saturate the beads surface area, Dynal particles (4.5 μm) were coated with different amounts of labelled antibody from Example A. Four 30 mg bead aliquots were washed with PBS and subsequently mixed in four separate tubes containing 530, 880, 952, and 988 μl of PBS containing 0.005% thimerosal. To their respective bead mixtures, 470, 120, 48, and 12 μl of 500 $\mu\text{g/ml}$ labelled antibody (Example A) was then added, and each mixture allowed to rotate over 1 hour. The beads were then separated from their solutions using a magnet. The supernatant was removed and saved for electrochemiluminescence analysis. 1 ml of PBS containing 3% BSA was then added to the beads which were allowed to rotate for 1 hour to block any remaining unoccupied areas on the particles. This washing and separation process was repeated once more, and afterwards the beads were stored in PBS with 3% BSA.

Example C

Analysis of the electrochemiluminescence generated from the

labelled beads (Example B) was done using a
ORIGEN Analyzer and reagents (Igen,
Incorporated) or could be performed in the
systems described in figure 4 and 5 above. The
5 analyzer first pumps the bead sample (150
 $\mu\text{g}/500\mu\text{l}$) to a flow cell containing a gold
working electrode, counter electrode, and a
Ag/AgCl reference electrode shown in Figure 6.
The beads are then allowed to settle (by
10 gravity) to the electrode surface where
electrochemical excitation is performed. The
settling time is varied depending on the density
of the beads and the distance it must travel to
reach the electrode - 370 μ being the longest
15 distance. Once the desired settling time is
done, the excitation takes place with the
subsequent generation of light. A
photomultiplier tube converts the light energy
to electrical energy that is processed by a
20 luminometer. Once the light has been collected
and analyzed, the existing sample is washed away
and the flow cell is cleaned and conditioned in
preparation for the next sample of beads to be
measured.

Example 1

Dynal particles (4.5 μm) (Table I, Example 1) were coated with labelled antibody prepared in Example A. 30 mg. were washed with
5 PBS and subsequently added to 988 μl of PBS containing 0.005% thimerosal. 12 μl of 500 $\mu\text{g/ml}$ labelled antibody (Example B) was then added to this particle mixture and allowed to rotate over 1 hour. The beads were then
10 separated from the solution using a magnet. The supernatant was removed and saved for electrochemiluminescence analysis. 1 ml of PBS containing 3% BSA was then added to the beads which were allowed to rotate for 1 hour to block
15 any remaining unoccupied areas on the particles. The beads were separated again. This washing and separation process was repeated once more, and afterwards the beads were stored in PBS with 3% BSA.

20 The results of an ECL analysis are set for in Table I.

Examples 2-9

The procedure described in Example 1 above was repeated using the particulate

magnetic solid phase supports described for Examples 2-9 of Table I.

Example 10

Glass beads (2 μ m) were coated with
5 labelled antibody prepared in Example A. 30 mg
of the beads were washed with PBS and
subsequently added to 988 μ l of PBS containing
0.005% thimerosal. 12 μ l of 500 μ g/ml labelled
10 antibody (prepared in Example B) was then added
to this bead mixture and allowed to rotate over
1 hour. The mixture was then centrifuged at
2500 rpm for 5 minutes. The supernatant was
removed and saved for electrochemiluminescence
analysis. 1 ml of PBS containing 3% BSA was
15 then added to the beads. The mixture was
centrifuged again to separate the beads. This
washing and centrifugation process was repeated
once more, and afterwards the beads were stored
in PBS with 3% BSA.

20 Examples 11-14

The procedure described in Example 10
above was repeated using the particulate
magnetic solid phase supports described for
Examples 2-9 of Table I.

TABLE I

EX NO.	PARTICLE TYPE MAGNETIC	MEAN DIAMETER (μ m)	DENSITY (g/ml)	MATERIAL	MAGNETIC Content (%)	RELATIVE ECL (%)	SHAPE
1	Dynal	4.5	1.5	PS/Fe ₃ O ₄	40	100.0	spherical
2	Polysciences	1.5-2.0	2.1	PS/FeO ₂	67	12.0	irregular aggregates
3	Advanced Magnetics	1.0	2.5	Silanized FeO ₂	NAV	0.2	irregular
4	Rhone-Poulenc	4.0	1.5	PSdvb/Fe ₃ O ₄	NAV	40.0	spherical
5		1.5-2.1	1.4	PS/Fe ₃ O ₄	23	11.0	irregular
6	Cortex	1.0-10.0	1.3	Cell/Fe ₃ O ₄	50	2.0	irregular
7		1.0-10.0	1.8	PACRO/"	60	2.0	irregular
8		1.0-25.0	1.2	XPACMD/"	33	2.0	irregular
9	Nickel	3.0	8.9	NI	100	3.0	irregular
Nonmagnetic							
10	Glass	8.0	2.4	soda lime	none	50.0	spherical
11		2.0	2.4	soda lime	none	200.0	spherical
12	Quartz	0.3-3.5	2.5	SiO ₂	none	26.0	irregular
13	Aluminum Silicate	0.2-6.0	2.5	AlO ₂ SiO ₂	none	6.0	irregular
14	Gold	1.0-2.0	19.3	Au	none	25.0	irregular

The result shown in Table I show that particle geometry and transmission properties for polystyrene containing Fe_2O_3 and soda lime glass provided significantly better and unexpected results when compared to other materials.

Although the invention has been described in conjunction with specific embodiments, it is evident that many alternatives and variations will be apparent to those skilled in the art in light of the foregoing disclosure. Accordingly, the invention is intended to embrace all of the alternatives and variations that fall with the spirit and scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method of detecting an analyte comprising:

- 5 a) providing a particulate solid phase support with a ligand containing luminescent metal label, said support and luminescent metal label having matched transmissive and emissive properties to enhance the emission from said metal label upon reaction with a coreactant;
- 10 b) contacting the solid phase support of step a) with an analyte of interest to form a luminescent labelled solid phase-analyte complex;
- 15 c) collecting said complex on an electrode; and
- 20 d) exposing said complex to electrochemical energy in the presence of a coreactant and measuring the resulting luminescence.
2. The method according to claim 1, wherein said electrogenerated chemiluminescence arises from a reaction of said label and said coreactant.
3. The method according to claim 1, wherein said coreactant is an amine.
4. The method according to claim 1, wherein said amine is tripropyl amine.

5. The method according to claim 1, wherein said ligand is an antibody, antigen or DNA probe.

6. The method according to claim 1, wherein said particulate solid phase support is selected from magnetic glass and magnetic polystyrene and is contoured to provide reproducible
5 presentation of said magnetic particulate support to an electrode surface.

7. The method according to claim 6, wherein said ligand is an antibody, antigen or DNA probe.

8. The method according to claim 8, wherein said metal luminescent label is an aromatic, heterocyclic nitrogen-compound containing ruthenium or osmium compound.

9. The method according to claim 4, wherein said metal luminescent label is formed from 4-methyl-4-N-succinimidyloxy-carbonylpropyl-2,2-bypyridine Ru(II).

10. An electrogenerated chemiluminescent solid phase support comprising:

a particulate substrate providing a reproducible surface presentation to an
5 electrode surface and having an exterior layer of a metal luminescent label, said substrate and metal label having matched transmissive and emissive properties to enhance the emission from said metal luminescent label upon reaction with
10 a coreactant.

11. The support to claim 10, wherein said metal label is attached to a ligand is an antibody, antigen or DNA probe.

12. The support according to claim 11, wherein said particulate substrate is selected from magnetic glass and magnetic polystyrene and is contoured to provide reproducible presentation
5 of said magnetic particulate substrate to an electrode surface.

13. The support according to claim 10, wherein said ligand is an antibody, antigen or DNA probe.

14. The support according to claim 10, wherein said luminescent label is succinimidyloxy carbonylpropyl-2,2-bypyridine.

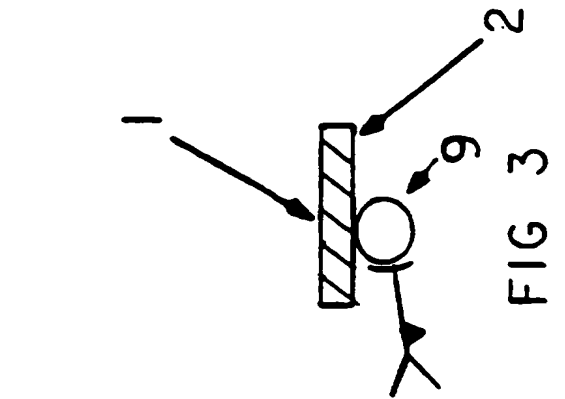


FIG. 1

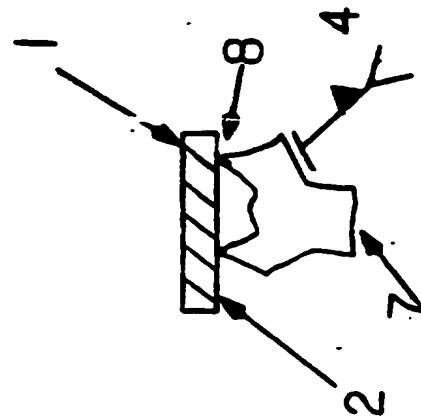


FIG. 2

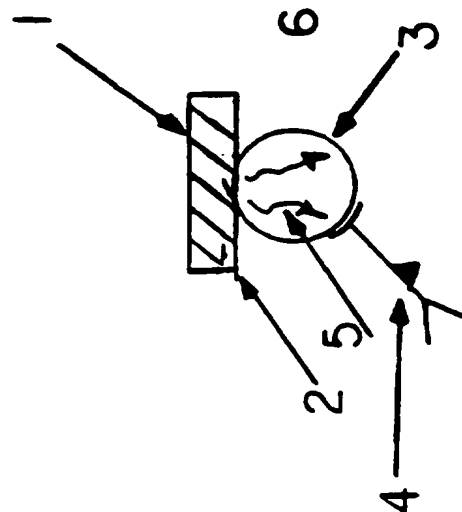
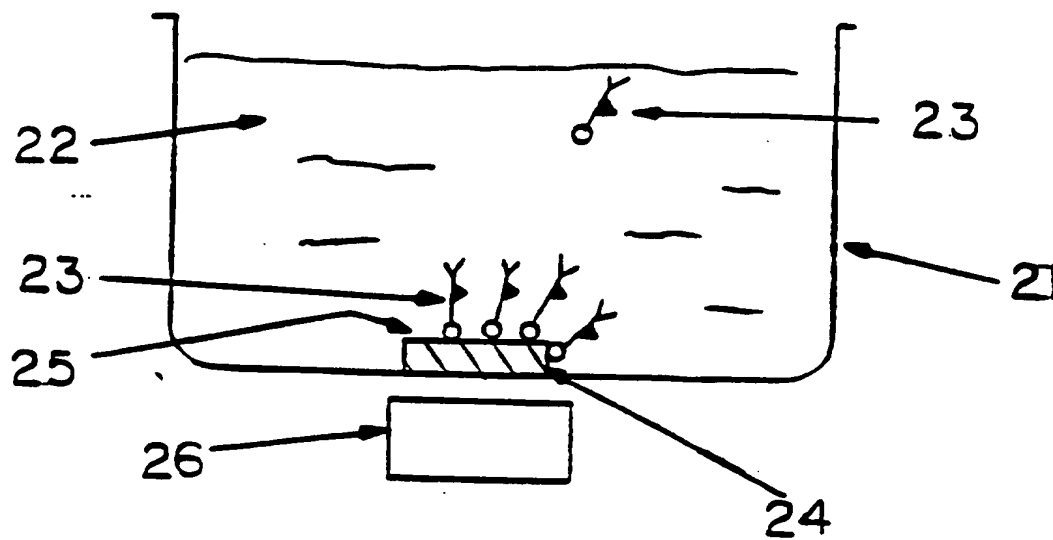
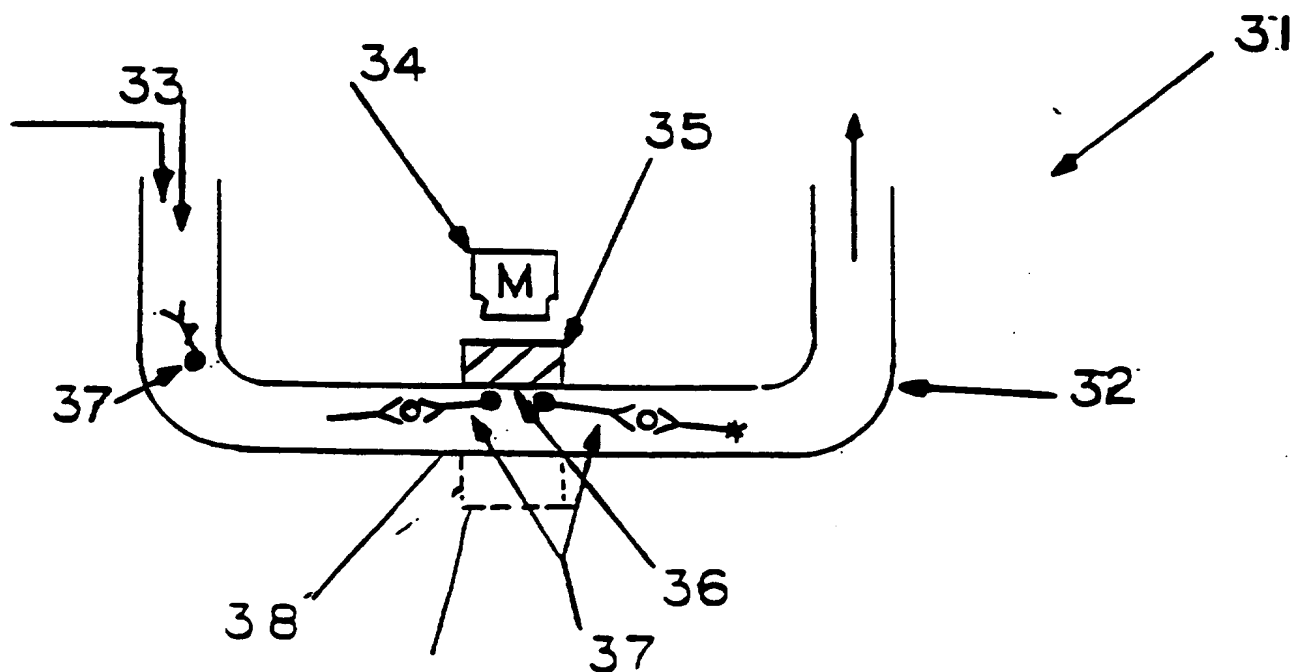
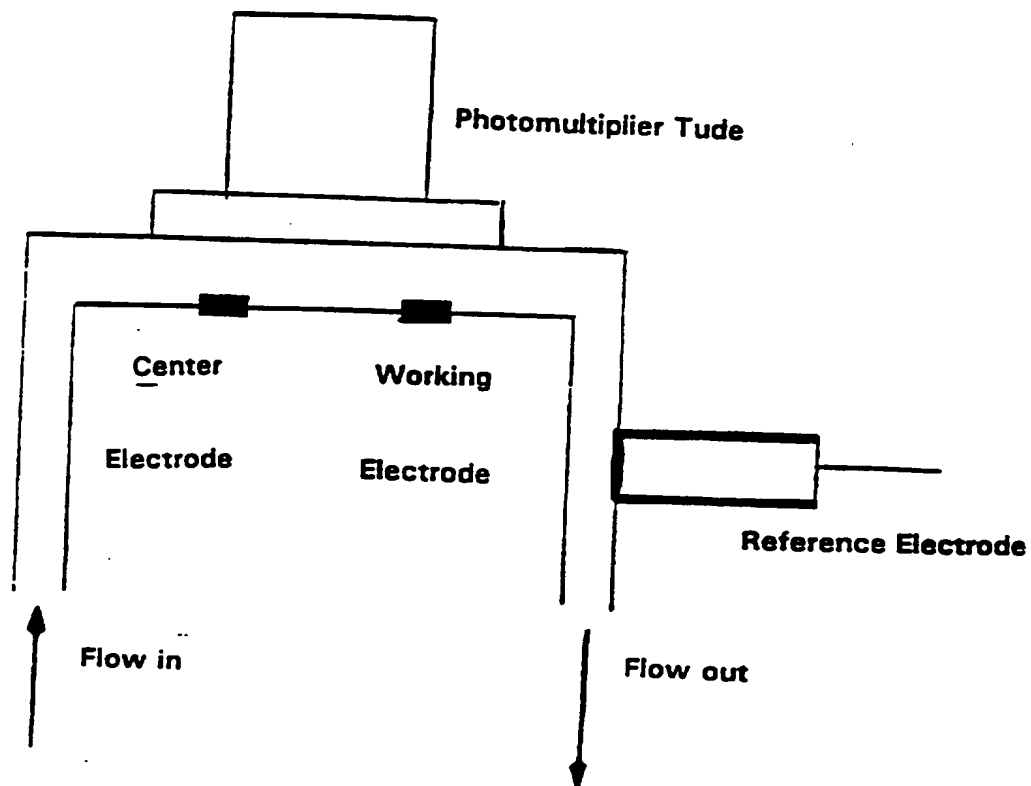


FIG. 3

FIG 4FIG 5

FIG 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/00493

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : G01N 33/543, 33/553

US CL : 436/518, 526

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/518, 526, 524, 528, 531, 533, 534

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN

search terms: electrochemiluminescent, particle, magnetic

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO, A, 90/05301 (IGEN, INC.) 17 May 1990, see pages 5, 6, 9-11 and 14.	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search

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